

EXPERIMENTAL STUDY

The effect of exercise on reelin level in the hippocampus of diabetic rats

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ABSTRACT

AIM: Diabetes is one of the most common diseases which can attenuate brain function by destroying hippocampus neurons, while reelin is a largely secreted extracellular matrix glycoprotein in the hippocampus causing synaptic plasticity, promoting postsynaptic structures and maturing neurons. The aim of this study was to assess the effect of exercise, as an external factor for neurogenesis in the brain, on reelin levels and memory improvement in diabetic rats.

METHOD: Thirty rats were randomly allocated into three groups; healthy sedentary, diabetic sedentary and diabetic exercise-trained. The experimental group was treadmill-exercised at speed 22 m/min for 1 hour, 5 days per week. Finally, spatial memory of rats was tested and reelin levels were measured.

RESULTS: The results showed that short-term exercise improved spatial memory in diabetic rats but had no effect on reelin levels in the hippocampus of diabetic rats.

CONCLUSION: Diabetes reduced the spatial memory without altering the reelin levels while exercise improved spatial memory without altering the reelin levels (*Fig. 4, Ref. 33*). Text in PDF www.elis.sk.

KEY WORDS: reelin, exercise, diabetes, memory, hippocampus.

Introduction

Type 1 diabetes, also known as insulin-dependent diabetes, occurs due to disturbance in sugar (glucose) metabolism. The lack of insulin secretion by the pancreas gland is the main underlying reason for type 1 diabetes (1). Several metabolic disorders such as diabetes mellitus (DM) may contrive molecular and cellular changes in central nervous system, especially in the hippocampus (2). Diabetes significantly reduces neuronal density in the dentate gyrus of hippocampus (3), which plays a crucial role in proper processing and spatial memory (4). On the other hand, learning and memory defects are more prevalent among diabetics compared to non-diabetic individuals (5). Type 1 diabetes leads to impaired learning, memory and cognition in animals

(6) and increases the risk of dementia, Alzheimer's disease and schizophrenia (7, 8).

Reelin is an extracellular glycoprotein that binds to the transmembrane receptors through APOE2 and VLDLR and activates Src tyrosine kinase. This cascade of enzymes is essential for long-term potentiation (LTP) and stimulation of NMDA receptors that play important roles in learning, memory, recalling information, and creating long-term potentiation (9). Reelin is necessary for proper development of the brain during the embryonic period and early organization of the cerebral cortex and cerebellum, as well as decent neuronal migration and radial glial cells migration into the suitable position (10). Reelin has an impact on the release of neurotransmitters in an adult brain, long-term memory formation and synaptic plasticity (11). This glycoprotein is generated by the hippocampus and cerebellum and is expressed for the whole life in adult brain (12).

It is well understood that neurogenesis is not a static process, it can be influenced by both external and internal factors and occurs in all parts of fetal brain and only in a limited area of adult brain including the hippocampus (13). On this basis, physical activity is an external factor that reduces blood glucose levels, increases cognitive performance and stimulates neurogenesis in the dentate gyrus of hippocampus (14, 15).

In general, there is much evidence about the relationship between exercise, neurogenesis, memory and mental function improvement in diabetic patients. Although, many studies have investigated the effect of reelin on neurogenesis in the hippocampus,

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Acknowledgement: This work was financially supported by Neurosciences Research Center, Tabriz University of Medical Sciences, Tabriz, Iran and Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran.

the aim of this study was to evaluate the effect of exercise on reelin levels in hippocampus and its effects on diabetes' complications, including spatial memory status.

Materials and methods

Animals

Thirty adult male Wistar rats weighing 250 ± 50 g, were obtained from the animal house of Tabriz University and housed in an animal room in groups of five per cage. They were maintained at 22 °C with free access to commercial rat chow and tap water. The animals were adapted to an inverse 12/12-hour light/dark cycle for 2 weeks prior to the exercise program. All experimental procedures employed as well as rat care and handling were approved by the Animal Care Committee of the Tabriz University of Medical Sciences and were in accordance with Experimental Animal Laboratory.

Induction of diabetes

Diabetes was induced in rats by intraperitoneal injection of 60 mg/kg streptozotocin (STZ) (Sigma, St Louis, MO) (16). Rats in the control groups received an intraperitoneal injection of an equal volume of citrate buffer instead of STZ. Blood samples were obtained from the tail vein and blood glucose concentrations were measured 48 hours after STZ administration and then confirmed by histopathological studies (17, 18).

Exercise method

Thirty rats were randomly allocated into three main groups (n = 10 each): healthy sedentary control (HS), diabetic sedentary control (DS) and diabetic exercise-trained group (DE15) which was subjected to running exercise for 15 days. The exercise protocol was chosen considering the capability to control the intensity

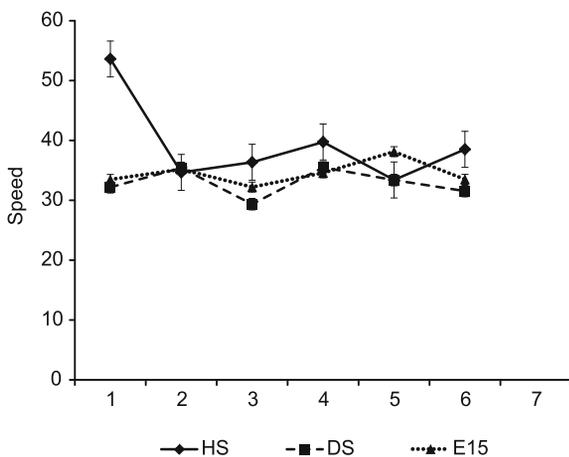


Fig. 1. Average swimming speed. Comparison of average swimming speed between healthy sedentary group (HS), diabetic sedentary group (DS) and diabetic exercise-trained group (DE15). There was no significant difference among different groups. All data were expressed as mean \pm SE.

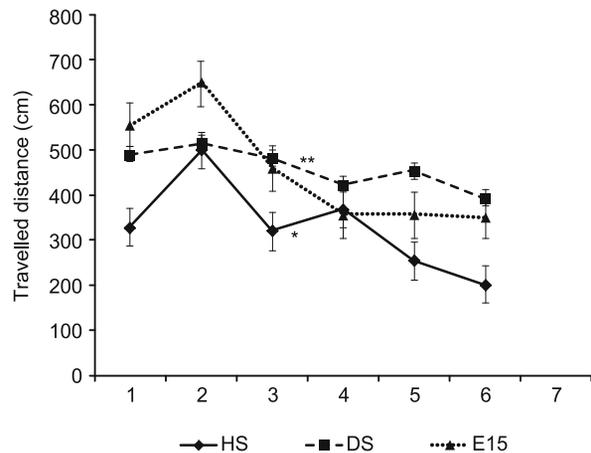


Fig. 2. Average total path. Comparison of average total path between healthy sedentary group (HS), diabetic sedentary group (DS) and diabetic exercise trained group (DE15). There was a significant difference between the healthy sedentary group (HS) and diabetic sedentary group (DS) (* p < 0.05). There was a significant difference between the healthy sedentary group (HS) and diabetic exercise-trained group (DE15) (** p < 0.05). All data were expressed as mean \pm SE.

of exercise, its duration, and timing. All rats in the trained group performed five exercise sessions per duration of 60 min at a 22 m/min pace on a 0 % slope, weekly. The first and last 5 minutes on the treadmill were considered as warm up and recovery periods. One week prior to the beginning of the training protocol, the rats were familiarized with treadmills in order to reduce the environmental stress.

Water maze method (MWM)

Rats in all study groups were trained on MWM with four trials per day for 6 days. The platform was hidden 2 cm below the surface of water. The time to reach the platform (latency), distance and speed were calculated (19).

ELISA assay

ELISA kit Reelin (E92775Ra purchased from Life Science Inc.) was used to measure the levels of the reelin protein according to the kit instruction

Results

The comparison of swimming speed among groups revealed no significant difference (Fig. 1). The path distance (path length) in the diabetic sedentary group did not show any significant difference in various days. However, a significant difference was observed between the healthy sedentary group (HS) and the diabetic sedentary group (DS) on days 3 and 6. In addition, there was a significant difference between the healthy sedentary group (HS) and diabetic exercise-trained group (DE15) on day 3 (Fig. 2).

During different days of observation, the rats in DS groups had no significant difference in terms of delayed detection of the platform (escape latency). The escape latency was significantly

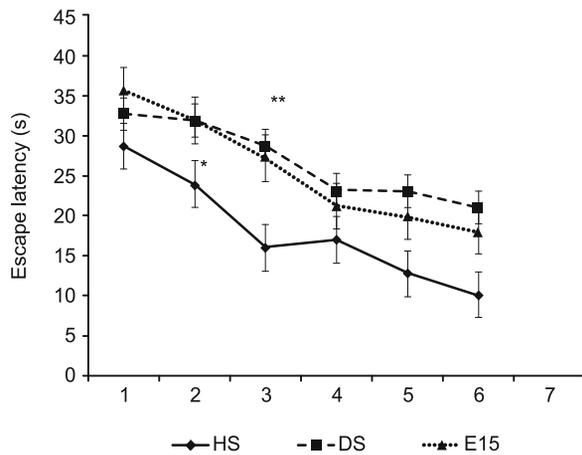


Fig. 3. Average total time. Comparison of average total time between healthy sedentary group (HS), diabetic sedentary group (DS) and diabetic exercise-trained group (DE15). There was a significant difference between the healthy sedentary group (HS) and diabetic sedentary group (DS) (* $p < 0.05$). There was a significant difference between the diabetic sedentary group (DS) and diabetic exercise-trained group (DE15) (** $p < 0.05$). All data were expressed as mean \pm SE.

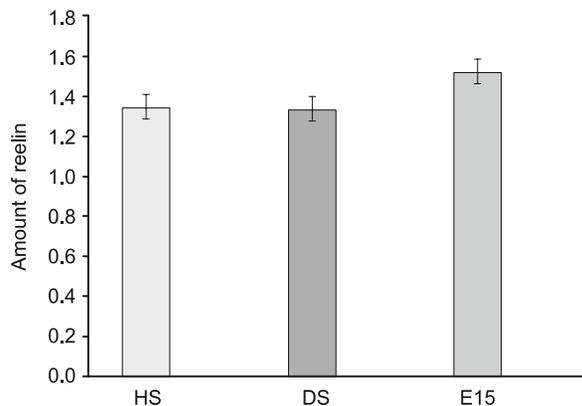


Fig. 4. Reelin levels. Comparison of reelin levels in the hippocampus of rats in healthy sedentary group (HS), diabetic sedentary group (DS) and diabetic exercise trained group (DE15). There was no significant difference between the results of different groups. All data were expressed as mean \pm SE.

different between the healthy sedentary group (HS) and diabetic sedentary group (DS) on day 2. Also, there was a significant difference between the diabetic sedentary control group (DS) and diabetic exercise-trained group (DE15) (Fig. 3).

ELISA test results showed no significant difference in reelin levels between study groups (Fig. 4).

Discussion

The results of present study showed a significant difference in terms of passed distance and escape latency between the healthy sedentary group (HS) and diabetic sedentary group (DS). Thus, we observed a significantly impaired memory function in the diabetic

animals. In this regard, previous investigations have shown that diabetes can cause a wide range of disorders in peripheral and central nervous system (20). Central neuropathy is a common complication of diabetes that is associated with disturbances in learning, memory and cognitive skills (21). These problems emerge over time and increase the risk of dementia and Alzheimer's disease in humans (22). The hippocampus is mostly vulnerable to DM I-induced hyperglycemia (23). Since, it has been considered as the main target of complications subsequent to diabetes, including cognitive dysfunction due to hippocampal neuron damage (24). Although the mechanisms of the damage to hippocampal neurons subsequent to diabetes are unknown, it has been shown that dendritic atrophy, decreased regulation of the glucocorticoid receptor, changes in expression of insulin-like growth factor receptor, reduction in insulin transmitter and induction of apoptosis are major pathologies that occur in neurons (25).

In the present study, the escape latency differed significantly between diabetic sedentary control group (DS) and diabetic exercise-trained group (DE15), which reveals an improvement in spatial memory in diabetic animals through short-term exercise. It should be noted that in the present study, memory was measured after exercise.

Our findings are consistent with previous studies that showed the effect of exercise on the brain and its activity, namely on increase in learning and memory of young rats (26). Similar to our results, it has been reported that treadmill exercise improves learning and memory performance in MWM (27) and proved that short-term treadmill running (for 10 days) increased learning in MWM and elevated the levels of epinephrine (28). In another study, 5 days of voluntary running, increased rats' learning, which was evaluated via MWM (29).

Previous studies summarized that exercise increases long-term potential (LTP) in different regions of the hippocampus and facilitates recovery of brain injury with upregulation of brain-derived neurotrophic factors such as BDNF, which enhances neurogenesis (30, 31). BDNF is an essential factor for Cajal Retzius cells located in the hippocampus. Cajal Retzius cells are also an important source for secretion of reelin (32). It is specified that reelin plays a role in memory and its role has been observed in patients with schizophrenia (31). Stranahan et al. found that reelin plays an important role in the improvement of hippocampus performance (33).

Although, previous studies hypothesized that exercise improves spatial memory in diabetic rats by increasing reelin, our result was inconsistent with literature. Exercise improved spatial memory in diabetic rats but not via reelin increase; it could have been performed through other above-mentioned mechanisms.

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Received September 11, 2019.

Accepted November 6, 2019.